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EXAMINER				
MCGARRY, SEAN				
ART UNIT		PAPER NUMBER		
1635				
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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

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Office Action Summary

Application No.

10/674,159

Applicant(s)

CHEN ET AL.

Examiner

SEAN MCGARRY

Art Unit

1635

Period for Reply -- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

- 1) ☒ Responsive to communication(s) filed on 20 November 2009.
- 2a) ☐ This action is **FINAL**. 2b) ☒ This action is non-final.
- 3) ☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

- 4) ☒ Claim(s) 39-48, 64-69, 94-97, 179-184 and 201 is/are pending in the application.
- 4a) Of the above claim(s) _____ is/are withdrawn from consideration.
- 5) ☐ Claim(s) _____ is/are allowed.
- 6) ☒ Claim(s) 39-48, 64-69, 94-97, 179-184 and 201 is/are rejected.
- 7) ☐ Claim(s) _____ is/are objected to.
- 8) ☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

- 9) ☐ The specification is objected to by the Examiner.
- 10) ☒ The drawing(s) filed on 23 September 2003 is/are: a) ☒ accepted or b) ☐ objected to by the Examiner.
- Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).
- Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).
- 11) ☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

- 12) ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).
- a) ☐ All b) ☐ Some * c) ☐ None of:
- ☐ Certified copies of the priority documents have been received.
 - ☐ Certified copies of the priority documents have been received in Application No. _____.
 - ☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

- 1) ☒ Notice of Filings/Copies Cited (PTO-502)
- 2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
- 3) ☐ Information Disclosure Statement(s) (PTO/SB/08)
Paper No(s)/Mail Date _____
- 4) ☐ Interview Summary (PTO-413)
Paper No(s)/Mail Date _____
- 5) ☐ Notice of Informal Patent Application
- 6) ☐ Other: _____

DETAILED ACTION

Continued Examination Under 37 CFR 1.114

A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on 11/20/2009 has been entered.

Claims 39-48, 64-69, 94-97, 179-184 and 201 are pending and are currently examined.

It is noted that the restriction among the sequences recited in the claims is now treated as a species requirement. It is further noted that the search was extended to the sequences recited in the claims where there was no anticipatory art found on the specific sequence recited, however see the new rejection under 35 U.S.C. 103(a) below.

This Official Action sets forth new grounds of rejection not previously made during the prosecution of the claimed invention. The new rejection under 35 U.S.C. 103(a) reconsiders the indication of allowability for the specific sequences recited in the claims.

Double Patenting

The nonstatutory double patenting rejection is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or improper timewise extension of the "right to exclude" granted by a patent and to prevent possible harassment by multiple assignees. A nonstatutory obviousness-type double patenting rejection is appropriate where the conflicting claims are not identical, but at least one examined application claim is not patentably distinct from the reference claim(s) because the examined application claim is either anticipated by, or would have been obvious over, the reference claim(s). See, e.g., *In re Berg*, 140 F.3d 1428, 46 USPQ2d 1226 (Fed. Cir. 1998); *In re Goodman*, 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); *In re Longi*, 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); *In re Van Ornum*, 686 F.2d 937, 214 USPQ 761 (CCPA 1982); *In re Vogel*, 422 F.2d 438, 164 USPQ 619 (CCPA 1970); and *In re Thorington*, 418 F.2d 528, 163 USPQ 644 (CCPA 1969).

A timely filed terminal disclaimer in compliance with 37 CFR 1.321(c) or 1.321(d) may be used to overcome an actual or provisional rejection based on a nonstatutory double patenting ground provided the conflicting application or patent either is shown to be commonly owned with this application, or claims an invention made as a result of activities undertaken within the scope of a joint research agreement.

Effective January 1, 1994, a registered attorney or agent of record may sign a terminal disclaimer. A terminal disclaimer signed by the assignee must fully comply with 37 CFR 3.73(b).

Claims 39-48, 64-69, 94-97, 179-184 and 201 are provisionally rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 201-204, 211-214, 221-224, 231-259, 262-264, 272-274, and 275-278 of copending Application No. 11/952,056. Although the conflicting claims are not identical, they are not patentably distinct from each other because both applications claims siRNA and shRNA compounds targeting influenza genes where the compounds are based on the same target sequences and where the compounds of the claims in each application overlap, embrace, are embraced, or are the same as the compounds claimed in the other application. The specification of both applications is the same where the differences of any claimed compounds fall within the definition of siRNAs and shRNAs disclosed in each specification.

This is a provisional obviousness-type double patenting rejection because the conflicting claims have not in fact been patented.

Specification

The disclosure was objected to because of the following informalities: Page 84 of the specification is blank.

The submission of a substitute page 84 is acknowledged. This page 84 has been entered into the application.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

(a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negated by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

Claims 39-48, 64-69, 94-97, 179-184 and 201 are rejected under 35 U.S.C. 103(a) as being unpatentable over Tuschl et al [US 2004/0259,247] and Beach et al [US 2002/0162126, cited by applicant] in view of Abe et al [European Journal of Pharmaceutical Sciences, Vol. 13:61-69, 2001, cited by applicant], Gittlin et al. [Nature Vol.418:430-434, 25 July 2002, cited by applicant], Brummelkamp et al [Science Vol.

296:550-553, 19 April 2002, cited by applicant], Paddison et al [Genes & Development Vol.16:948-958, 2002], and Elbashir et al [Methods Vol.26:199-213, 2002].

The invention is as clearly set forth in the claims.

Tuschl et al have taught the use of siRNA and have taught that siRNA may be used to inhibit viral genes. Tuschl teaches, for example:

[0008] The object underlying the present invention is to provide novel agents capable of mediating target-specific RNA interference or other target-specific nucleic acid modifications such as DNA methylation, said agents having an improved efficacy and safety compared to prior art agents.

[0009] The solution of this problem is provided by an isolated double-stranded RNA molecule, wherein each RNA strand has a length from 19-25, particularly from 19-23 nucleotides, wherein said RNA molecule is capable of mediating target-specific nucleic acid modifications, particularly RNA interference and/or DNA methylation. Preferably at least one strand has a 3'-overhang from 1-5 nucleotides, more preferably from 1-3 nucleotides and most preferably 2 nucleotides. The other strand may be blunt-ended or has up to 6 nucleotides 3' overhang. Also, if both strands of the dsRNA are exactly 21 or 22 nt, it is possible to observe some RNA interference when both ends are blunt (0 nt overhang). The RNA molecule is preferably a synthetic RNA molecule which is substantially free from contaminants occurring in cell extracts, e.g. from Drosophila embryos. Further, the RNA molecule is preferably substantially free from any non-target-specific contaminants, particularly non-target-specific RNA molecules e.g. from contaminants occurring in cell extracts.

[0010] Further, the invention relates to the use of isolated double-stranded RNA molecules, wherein each RNA strand has a length from 19-25 nucleotides, for mediating, target-specific nucleic acid modifications, particularly RNAi, in mammalian cells, particularly in human cells.

[0011] Surprisingly, it was found that synthetic short double-stranded RNA molecules particularly with overhanging 3'-ends are sequence-specific mediators of RNAi and mediate efficient target-RNA cleavage, wherein the cleavage site is located near the center of the region spanned by the guiding short RNA.

[0012] Preferably, each strand of the RNA molecule has a length from 20-22 nucleotides (or 20-25 nucleotides in mammalian cells), wherein the length of each strand may be the same or different. Preferably, the length of the 3'-overhang reaches from 1-3 nucleotides, wherein the length of the overhang may be the same or different for each strand. The RNA-strands preferably have 3'-hydroxyl groups. The 5'-terminus preferably comprises a phosphate, diphosphate, triphosphate or hydroxyl group. The most effective dsRNAs are composed of two 21 nt strands which are paired such that 1-3, particularly 2 nt 3' overhangs are present on both ends of the dsRNA.

[0013] The target RNA cleavage reaction guided by siRNAs is highly sequence-specific. However, not all positions of a siRNA contribute equally to target recognition. Mismatches in the center of the siRNA duplex are most critical and essentially abolish target RNA cleavage. In contrast, the 3' nucleotide of the siRNA strand (e.g. position 21) that is complementary to the single-stranded target RNA, does not contribute to specificity of the target recognition. Further, the sequence of the unpaired 2-nt 3' overhang of the siRNA strand with the same polarity as the target RNA is not critical for target RNA cleavage as only the antisense siRNA strand guides target recognition. Thus, from the single-stranded overhanging nucleotides only the penultimate position of the antisense siRNA (e.g. position 20) needs to match the targeted sense mRNA.

[0014] Surprisingly, the double-stranded RNA molecules of the present invention exhibit a high in vivo stability in serum or in growth medium for cell cultures. In order to further enhance the stability, the 3'-overhangs may be stabilized against degradation, e.g. they may be selected such that they consist of purine nucleotides, particularly adenosine or guanosine nucleotides. Alternatively, substitution of pyrimidine nucleotides by modified analogues, e.g. substitution of uridine 2 nt 3' overhangs by 2'-deoxythymidine is tolerated and does not affect the efficiency of RNA interference. The absence of a 2' hydroxyl significantly enhances the nuclease resistance of the overhang in tissue culture medium.

[0015] In an especially preferred embodiment of the present invention the RNA molecule may contain at least one modified nucleotide analogue. The nucleotide analogues may be located at positions where the target-specific activity, e.g. the RNAi mediating activity is not substantially effected, e.g. in a region at the 5'-end and/or the 3'-end of the double-stranded RNA molecule. Particularly, the overhangs may be stabilized by incorporating modified nucleotide analogues.

[0016] Preferred nucleotide analogues are selected from sugar- or backbone-modified ribonucleotides. It should be noted, however, that also nucleobase-modified ribonucleotides, i.e. ribonucleotides, containing a non-naturally occurring nucleobase instead of a naturally occurring nucleobase such as uridines or cytidines modified at the 5-position, e.g. 5-(2-amino)propyl uridine, 5-bromo uridine; adenosines and guanosines modified at the 8-position, e.g. 8-bromo guanosine; deaza nucleotides, e.g. 7-deaza-adenosine; O- and N-alkylated nucleotides, e.g. N6-methyl adenosine are suitable. In preferred sugar-modified ribonucleotides the 2'OH-group is replaced by a group selected from H, OR, R, halo, SH, SR, NH.sub.2, NHR, NR.sub.2 or CN, wherein R is C.sub.1-C.sub.6 alkyl, alkenyl or alkynyl and halo is F, Cl, Br or I. In preferred backbone-modified ribonucleotides the phosphoester group connecting to adjacent ribonucleotides is replaced by a modified group, e.g. of phosphothioate group. It should be noted that the above modifications may be combined.

[0017] The sequence of the double-stranded RNA molecule of the present invention has to have a sufficient identity to a nucleic acid target molecule in order to mediate target-specific RNAi and/or DNA methylation. Preferably, the sequence has an identity of at least 50%, particularly of at least 70% to the desired target molecule in the double-stranded portion of the RNA molecule. More preferably, the identity is at least 85% and most preferably 100% in the double-stranded portion of the RNA molecule. The identity of a double-stranded RNA molecule to a

predetermined nucleic acid target molecule, e.g. an mRNA target molecule may be determined as follows: $1 \text{ I} = n \text{ L} \cdot \text{times} \cdot 100$

[0020] The double-stranded RNA molecule of the invention may be prepared by a method comprising the steps:

[0021] (a) synthesizing two RNA strands each having a length from 19-25, e.g. from 19-23 nucleotides, wherein said RNA strands are capable of forming a double-stranded RNA molecule, wherein preferably at least one strand has a 3'-overhang from 1-5 nucleotides,

[0022] (b) combining the synthesized RNA strands under conditions, wherein a double-stranded RNA molecule is formed, which is capable of mediating target-specific nucleic acid modifications, particularly RNA interference and/or DNA methylation.

[0028] Preferably the contacting step (a) comprises introducing the double-stranded RNA molecule into a target cell, e.g. an isolated target cell, e.g. in cell culture, a unicellular microorganism or a target cell or a plurality of target cells within a multicellular organism. More preferably, the introducing step comprises a carrier-mediated delivery, e.g. by liposomal carriers or by injection.

[0030] The target gene to which the RNA molecule of the invention is directed may be associated with a pathological condition. For example, the gene may be a pathogen-associated gene, e.g. a viral gene, a tumor-associated gene or an autoimmune disease-associated gene. The target gene may also be a heterologous gene expressed in a recombinant cell or a genetically altered organism. By determining or modulating, particularly, inhibiting the function of such a gene valuable information and therapeutic benefits in the agricultural field or in the medicine or veterinary medicine field may be obtained.

[0031] The dsRNA is usually administered as a pharmaceutical composition. The administration may be carried out by known methods, wherein a nucleic acid is introduced into a desired target cell in vitro or in vivo. Commonly used gene transfer techniques include calcium phosphate, DEAE-dextran, electroporation and microinjection and viral methods (Graham, F. L. and van der Eb, A. J. (1973) *Virology* 52, 456; McCutchan, J. H. and Pagano, J. S. (1968), *J. Natl. Cancer Inst.* 41, 351; Chu, G. et al (1987), *Nucl. Acids Res.* 15, 1311; Fraley, R. et al. (1980), *J. Biol. Chem.* 255, 10431; Capecchi, M. R. (1980), *Cell* 22, 479). A recent addition to this arsenal of techniques for the introduction of DNA into cells is the use of cationic liposomes (Feigner, P. L. et al. (1987), *Proc. Natl. Acad. Sci USA* 84, 7413). Commercially available cationic lipid formulations are e.g. Tfx 50 (Promega) or Lipofectamin 2000 (Life Technologies).

[0032] Thus, the invention also relates to a pharmaceutical composition containing as an active agent at least one double-stranded RNA molecule as described above and a pharmaceutical carrier. The composition may be used for diagnostic and for therapeutic applications in human medicine or in veterinary medicine.

[0033] For diagnostic or therapeutic applications, the composition may be in form of a solution, e.g. an injectable solution, a cream, ointment, tablet, suspension or the like. The composition may be administered in any suitable way, e.g. by injection, by oral, topical, nasal, rectal application etc. The carrier may be any suitable pharmaceutical carrier. Preferably, a carrier is used, which is capable of increasing the efficacy of the RNA molecules to enter the target-cells. Suitable examples of such carriers are liposomes, particularly cationic liposomes. A further preferred administration method is injection.

[0035] Thus, a further subject matter of the invention is a eukaryotic cell or a eukaryotic non-human organism exhibiting a target gene-specific knockout phenotype comprising an at least partially deficient expression of at least one endogenous target gene wherein said cell or organism is transfected with at least one double-stranded RNA molecule capable of inhibiting the expression of at least one endogenous target gene or with a DNA encoding at least one double stranded RNA molecule capable of inhibiting the expression of at least one endogenous target gene. It should be noted that the present invention allows a target-specific knockout of several different endogenous genes due to the specificity of RNAi.

Tuschl et al have provided details on the design of siRNA compound which can be used to target a gene of known sequence where it has been taught that such compound can be used to inhibit viruses. Tuschl et al have also taught [See paragraphs 8 and 148, for example] that siRNA compound are more effective than prior art compound such as antisense and ribozymes. It is clear for the disclosure of Tuschl that one in the art would have a reasonable expectation of making siRNA compounds to any desired target gene sequence.

Beach et al also teach siRNA and teach shRNA compounds that may be expressed from a vector. Beach teaches, for example:

[0014] In certain preferred embodiments, the target gene is an endogenous gene of the cell. In other embodiments, the target gene is an heterologous gene relative to the genome of the cell, such as a pathogen gene, e.g., a viral gene.

[0017] In certain preferred embodiments, the length of the dsRNA is at least 20, 21 or 22 nucleotides in length, e.g., corresponding in size to RNA products produced by Dicer-dependent

cleavage. In certain embodiments, the dsRNA construct is at least 25, 50, 100, 200, 300 or 400 bases. In certain embodiments, the dsRNA construct is 400-800 bases in length.

[0018] In certain preferred embodiments, expression of the target gene is attenuated by at least 5 fold, and more preferably at least 10, 20 or even 50 fold, e.g., relative to the untreated cell or a cell treated with a dsRNA construct which does not correspond to the target gene. Tuschl and beach et al do not teach specifically targeting influenza or the np gene of influenza. They do not specifically teach targeting conserved regions of a viral gene, for example.

[0037] The dsRNA construct may comprise one or more strands of polymerized ribonucleotide. It may include modifications to either the phosphate-sugar backbone or the nucleoside. The double-stranded structure may be formed by a single self-complementary RNA strand or two complementary RNA strands. RNA duplex formation may be initiated either inside or outside the cell. The dsRNA construct may be introduced in an amount which allows delivery of at least one copy per cell. Higher doses of double-stranded material may yield more effective inhibition. Inhibition is sequence-specific in that nucleotide sequences corresponding to the duplex region of the RNA are targeted for genetic inhibition. dsRNA constructs containing a nucleotide sequences identical to a portion of the target gene is preferred for inhibition. RNA sequences with insertions, deletions, and single point mutations relative to the target sequence have also been found to be effective for inhibition. Thus, sequence identity may be optimized by alignment algorithms known in the art and calculating the percent difference between the nucleotide sequences. Alternatively, the duplex region of the RNA may be defined functionally as a nucleotide sequence that is capable of hybridizing with a portion of the target gene transcript.

[0084] As used herein, the term "vector" refers to a nucleic acid molecule capable of transporting another nucleic acid to that it has been linked. One type of vector is a genomic integrated vector, or "integrated vector", which can become integrated into the chromosomal DNA of the host cell. Another type of vector is an episomal vector, i.e., a nucleic acid capable of extra-chromosomal replication. Vectors capable of directing the expression of genes to that they are operatively linked are referred to herein as "expression vectors". In the present specification, "plasmid" and "vector" are used interchangeably unless otherwise clear from the context.

[0120] The cell with the target gene may be derived from or contained in any organism (e.g., plant, animal, protozoan, virus, bacterium, or fungus). The dsRNA construct may be synthesized either in vivo or in vitro. Endogenous RNA polymerase of the cell may mediate transcription in vivo, or cloned RNA polymerase can be used for transcription in vivo or in vitro. For generating double stranded transcripts from a transgene in vivo, a regulatory region may be used to transcribe the RNA strand (or strands).

[0122] The cell with the target gene may be derived from or contained in any organism. The organism may a plant, animal, protozoan, bacterium, virus, or fungus. The plant may be a monocot, dicot or gymnosperm; the animal may be a vertebrate or invertebrate. Preferred microbes are those used in agriculture or by industry, and those that are pathogenic for plants or animals. Fungi include organisms in both the mold and yeast morphologies.

[0128] The target gene may be a gene derived from the cell, an endogenous gene, a transgene, or a gene of a pathogen which is present in the cell after infection thereof. Depending on the particular target gene and the dose of double stranded RNA material delivered, the procedure may provide partial or complete loss of function for the target gene. Lower doses of injected material and longer times after administration of dsRNA may result in inhibition in a smaller fraction of cells. Quantitation of gene expression in a cell may show similar amounts of inhibition at the level of accumulation of target mRNA or translation of target protein.

[0133] The dsRNA construct may comprise one or more strands of polymerized ribonucleotide. It may include modifications to either the phosphate-sugar backbone or the nucleoside. For example, the phosphodiester linkages of natural RNA may be modified to include at least one of a nitrogen or sulfur heteroatom. Modifications in RNA structure may be tailored to allow specific genetic inhibition while avoiding a general panic response in some organisms which is generated by dsRNA. Likewise, bases may be modified to block the activity of adenosine deaminase. The dsRNA construct may be produced enzymatically or by partial/total organic synthesis, any modified ribonucleotide can be introduced by in vitro enzymatic or organic synthesis.

[0135] The double-stranded structure may be formed by a single self-complementary RNA strand or two complementary RNA strands. RNA duplex formation may be initiated either inside or outside the cell. The RNA may be introduced in an amount which allows delivery of at least one copy per cell. Higher doses (e.g., at least 5, 10, 100, 500 or 1000 copies per cell) of double-stranded material may yield more effective inhibition; lower doses may also be useful for specific applications. Inhibition is sequence-specific in that nucleotide sequences corresponding to the duplex region of the RNA are targeted for genetic inhibition.

[0137] 100% sequence identity between the RNA and the target gene is not required to practice the present invention. Thus the invention has the advantage of being able to tolerate sequence variations that might be expected due to genetic mutation, strain polymorphism, or evolutionary divergence.

[0138] The dsRNA construct may be synthesized either in vivo or in vitro. Endogenous RNA polymerase of the cell may mediate transcription in vivo, or cloned RNA polymerase can be used for transcription in vivo or in vitro. For transcription from a transgene in vivo or an expression construct, a regulatory region (e.g., promoter, enhancer, silencer, splice donor and acceptor, polyadenylation) may be used to transcribe the dsRNA strand (or strands). Inhibition may be targeted by specific transcription in an organ, tissue, or cell type; stimulation of an environmental condition (e.g., infection, stress, temperature, chemical inducers); and/or engineering transcription at a developmental stage or age. The RNA strands may or may not be polyadenylated; the RNA strands may or may not be capable of being translated into a polypeptide by a cell's translational apparatus. The dsRNA construct may be chemically or enzymatically synthesized by manual or automated reactions. The dsRNA construct may be synthesized by a cellular RNA polymerase or a bacteriophage RNA polymerase (e.g., T3, T7, SP6). The use and production of an expression construct are known in the art 32,33,34 (see also WO 97/32016; U.S. Pat. Nos. 5,593,874, 5,698,425, 5,712,135, 5,789,214, and 5,804,693; and

the references cited therein). If synthesized chemically or by in vitro enzymatic synthesis, the RNA may be purified prior to introduction into the cell. For example, RNA can be purified from a mixture by extraction with a solvent or resin, precipitation, electrophoresis, chromatography or a combination thereof. Alternatively, the dsRNA construct may be used with no or a minimum of purification to avoid losses due to sample processing. The dsRNA construct may be dried for storage or dissolved in an aqueous solution. The solution may contain buffers or salts to promote annealing, and/or stabilization of the duplex strands.

[0139] Physical methods of introducing nucleic acids include injection of a solution containing the dsRNA construct, bombardment by particles covered by the dsRNA construct, soaking the cell or organism in a solution of the RNA, or electroporation of cell membranes in the presence of the dsRNA construct. A viral construct packaged into a viral particle would accomplish both efficient introduction of an expression construct into the cell and transcription of dsRNA construct encoded by the expression construct. Other methods known in the art for introducing nucleic acids to cells may be used, such as lipid-mediated carrier transport, chemical mediated transport, such as calcium phosphate, and the like. Thus the dsRNA construct may be introduced along with components that perform one or more of the following activities: enhance RNA uptake by the cell, promote annealing of the duplex strands, stabilize the annealed strands, or other-wise increase inhibition of the target gene.

Beach et al have shown, for example that it was well within the skill of the artisan at the time of invention to design shRNA compounds based on siRNA sequences such that the inhibitory compound may be expressed in cells.

Both beach and Tuschl have taught that sequence variability can be tolerated at specified positions in a siRNA or shRNA compound.

Neither Tuschl et al or Beach et al have taught the inhibition of influenza genes or the specific sequences recited in the claims.

Abe et al have taught the inhibiting of influenza virus via the use of antisense oligonucleotides targeting various genes of the influenza genome. It has been taught, for example to target the PB1, PB2, PA and NP genes of influenza. Abe et teach the use of liposomes. Abe et al have shown a protective effect of antisense inhibition of

influenza in mice that were provided antisense via i.v. administration showing reduced influenza proliferation in lungs, reduced lung damage and increased survival rates. Abe et al have therefore demonstrated nucleic acid inhibition as a viable means for protecting against the influenza virus.

Abe et al have therefore shown that targeting influenza via a nucleic acid based inhibitor such as antisense was known in the art and has been shown to inhibit influenza in vivo. One in the art would clearly substitute siRNA for the antisense compounds of the prior art since the prior art has shown siRNA to be more effective nucleic acid expression inhibitor.

Gitlin et al have shown the inhibition of polio virus genes via the use of siRNA compounds. Gitlin et al have shown that siRNA can provide a therapeutic strategy against human viruses. Gitlin et al have also taught that improved strategy of inhibiting or providing cellular immunity to human viruses, especially RNA viruses will have to contend with their variability due to high mutation rates. They teach that using dsRNA directed to multiple conserved RNA target sequences will provide for the aforementioned.

Gitlin therefore has shown that indeed siRNA compound function in mammalian cells to inhibit viral replication. Gitlin et al have also provided teaching and motivation to target multiple conserved regions of viral genes to contend with variability due to high mutation rates (see page 433, right column first full paragraph and the last paragraph at page 434, for example).

Brummelkamp et al and Paddison et al have both taught the use of vectors utilizing polIII promoters for the effective expression of siRNA and shRNA compounds in vivo.

Elbashir et al have provided guidelines for the design of siRNA compounds. The target sequences of the instant invention fall squarely within the parameters taught by Elbashir et al.

Since one in the art has been apprised of the importance of targeting conserved regions of viral genes and since it was well within the skill of one in the art at the time of invention to determine conserved regions utilizing well know computer programs, for example, it would then have been simple to the screen those conserved regions for siRNA targeting regions utilizing the parameters set forth by Elbashir et al. The instant specification indicates that the siRNA target sequences were indeed selected by known means (see page 88-89 of the instant specification). Since the target sequences of the instant invention fall within the parameters set forth by the prior art for siRNA design it is unclear how the sequences recited in the instant claims define over the prior art. Elbashir et al have clearly taught to make such target sequences where there is a high probability of screening those for optimal inhibition. There do not appear to be any unexpected properties attached to the recited compounds [where each sequence recited in the instant claims includes a genus of compound based on the recited sequence].

The art taken as a whole has therefore shown that there was a need in the art to inhibit influenza virus which was well known in the art to be a problem in the human

population, for example. The art has shown the inhibition of influenza targeting various influenza genes via antisense compounds where Beach et al and Tuschl et al both taught that siRNA is a more effective and safer means of gene inhibition than antisense. Gitlin et al have demonstrated the inhibition of human viral genes via siRNA. Gitlin has also taught to target conserved regions of viral genes to contend with high mutation rates. One in the art would clearly have looked to use a more effective compound in the inhibition of influenza. The prior art has also provided teachings on the design of siRNA based on target sequence selection where the instantly recited sequences are based on target sequences that fall squarely within the parameters set forth by the prior art. The art has taught that known means of delivery and nucleic acid modifications can be used for/in siRNA compounds. The art has taught that siRNA and shRNA compounds can be delivered via vectors and specifically teaches the use of polIII promoters as an effective means of providing siRNA and shRNA compounds. The art has also taught that to better provide protection from viral infection, that multiple conserved regions of the viral genes should be targeted. The prior art also shows that siRNA has the capacity to inhibit gene expression by many fold where the inhibition levels are optimized via siRNA selection, dose, duration and delivery means.

The invention as a whole would therefore have been *prima facie* obvious to one in the art at the time the invention was made.

Conclusion

The following art made of record and not relied upon, but is considered pertinent to applicant's disclosure. US 7,199,109; US7,304,042; and US7,288,531 claim and/or disclose subject matter claimed herein.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to SEAN MCGARRY whose telephone number is (571)272-0761. The examiner can normally be reached on M-Th (6:00-4:30).

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Heather Calamita can be reached on (571) 272-2876. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

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Application/Control Number: 10/674,159
Art Unit: 1635

Page 17

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